REMARKS

Reconsideration is requested.

Claims 1, 3-7, 10, 14, 15, 17-24, 27-29 and 32-35 are pending.

Claims 1, 3-7, 10, 14, 15, 17-24 and 27-29 have been canceled above, without prejudice, to reduce the issues for appeal. Claims 32-35 will be pending upon entry of the present Amendment. The present Amendment should be entered pursuant to Rule 116(b)(1) ("An Amendment may be made canceling claims" after a final rejection). The applicants have complied with the suggestion of MPEP § 714.12 prior to the final rejection by presenting claims varying in scope and the applicants now wish to cancel the above-noted claims to at least reduce the issues for appeal. The present Amendment does not raise new issues requiring further search and/or consideration. According to MPEP § 714.13, entry of the present Amendment is not contingent on a showing required by Rule 116(b)(3).

Claims 32-35 will be pending upon entry of the present Amendment.

The claims define methods of increasing sialylation and/or N-glycan charge of a glycolylated protein expressed by a glutamine auxotrophic human cell. The applicants have discovered, and the application exemplifies, that the elevated rate of synthesis of a sialylated protein when a transfected cell according to the claimed invention is grown in a glutamine-free media. The Examples further demonstrate the reduced concentration of ammonia produced by a transfected cell according to the claimed invention in a glutamine-free media as compared to the concentration of ammonia a control cell not containing the second exogenous DNA sequence produces in media containing

glutamine. The applicants have unexpectedly discovered that glutamine-auxotropic human cells are able to increase sialylation and/or N-glycan charge of a glycosylated protein expressed by the cell if the cell is transfect with an exogenous DNA sequence encoding a glutamine synthetase and grown or cultured in a glutamine-free media. The cells of the disclosure reduce the concentration of ammonia in cell culture or media when grown in a glutamine-free media or culture which allows for a greater rate of protein synthesis and increased maximum product concentration (see for example, page 25, lines 3-7 of the specification) and increased degree of sialylation of the expressed glycosylated product (see for example, page 27, lines 2-5 of the specification).

There is no suggestion in the cited art or reasonable expectation from the cited art that a glutamine auxotrophic cell line, as required by the present claims, i.e., a cell line that by definition of the term auxotrophic is unable to synthesize glutamine, could grow in a glutamine-free media, as required by the present claims. There was no reasonable expectation from the cited art that a glutamine auxotrophic cell line, which has been transfected with a DNA sequence encoding a glutamine synthetase, could be grown in a glutamine free media, as required by the claims.

Further, the claimed invention is contrary to the suggestions of the cited art.

Specifically, the Examiner cites Gawlitzek (Biotechnology and Bioengineering, Vol. 68, No. 6, June 2000, pp 637-646) as a new reference in the Office Action of January 15, 2009 to allegedly

> "teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine

> metabolism and the thermal degradation of glutamine. Gawlitzek et al. further teaches that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of TNFR-IgG. Thus, the reference provides a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein in a cell without adding glutamine. [The reference] teach[es] that increasing the N-glycan of a glycosylated protein, i.e. increasing the ratio of glycosylation of a glycosylated protein, decides [sic, determines?] the activity of the protein." See pages 5-6 of the Office Action dated January 28, 2009.

As stated by the Examiner, Gawlitzek teaches that

> "[a]mmonium is mainly a byproduct of glutamine metabolism and the thermal degradation of glutamine" See page 637 of Gawlitzek, right column.

As also suggested by the Examiner, Gawlitzek teaches that

> "[a]s ammonium increased from 1 to 5 mM, a concomitant decrease of up to 40% was observed in terminal galactosylation and sialylation of the [TNFR-IgG] molecule." See Abstract of Gawlitzek.

The Examiner's conclusion drawn from, and reliance on, the reference however is contrary to Gawlitzek. Specifically, the Examiner concludes that Gawlitzek allegedly provides a suggestion for increasing sialylation and/or N-glycan charge of a glycosylated protein in a cell by limiting or eliminating glutamine. In fact, the Discussion of Gawlitzek taken with the results of Figure 7 of Gawlitzek, for example, indicate that limiting or eliminating glutamine in the model CHO cell of Gawlitzek will not effect terminal galactosylation and sialylation.

More specifically, Gawlitzek concluded as follows:

> "The results presented here strongly suggest that ammonium inhibits galactosylation and sialylation of TNFR-IgG N-glycans by pH-regulated mechanisms. We hypothesize that ammonium decreases α2,3-

> sialyltransferase and β1,4-galactosyltransferase activities by increasing the pH of the *trans*-Golgi compartment." See page 644, right column, first two sentences of the last paragraph, of Gawlitzek.

Gawlitzek concludes therefore that ammonium decreases transferase enzyme activity related to terminal galactosylation and sialylation due to an increase in pH. The following reproduction of Figure 8 of Gawlitzek (see page 644 of the reference) demonstrates that α2,3-sialyltransferase and β1,4-galactosyltransferase activities are pH dependent.

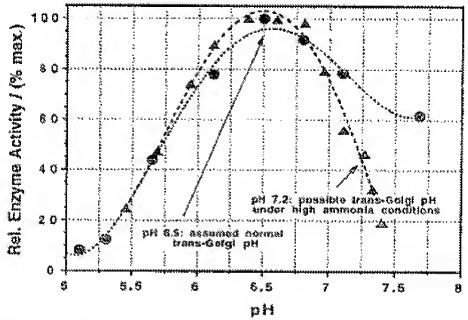
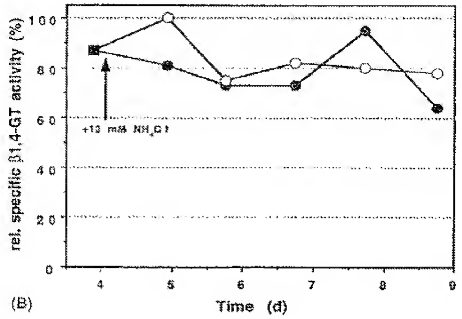


Figure 8. The pH profile of CHO α2,3-ST (▲) and β1,4-GT (●) activity.

The following reproduction of Figure 7 of Gawlitzek however demonstrates that similar enzyme activities were found when comparing cells cultivated under low and high ammonium concentrations (see also the description of Figure 7 on page 643 of Gawlitzek):



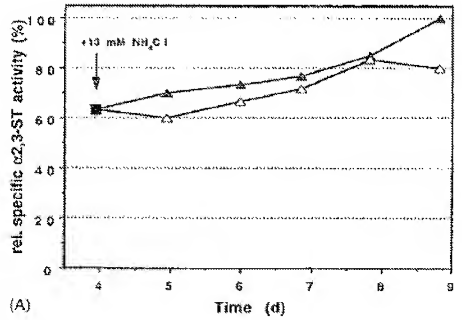


Figure 7. Enzyme activities of β1,4-galactosyltransferase and α2,3-sialyltransferase in CHO cells cultivated under control (−Gln) and high-ammonium (−Gln/+13 m*M* NH_4Cl) conditions. On day 4, 13 m*M* NH_4Cl was added after the enzyme samples were taken.

Gawlitzek also demonstrates with the following Figure 6 that similar mRNA levels of the enzymes were found when comparing the CHO cells cultivated under low or high ammonium concentrations.

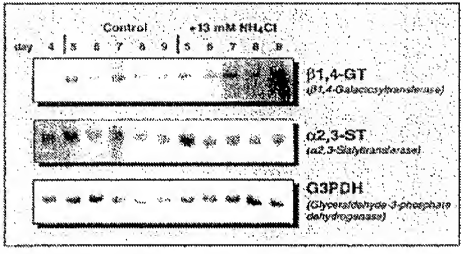


Figure 6. Northern blot analysis of β1,4-galactosyltransferase and α2,3-sialyltransferase mRNA in CHO cells cultivated under control (−Gln) and high-ammonium (−Gln/+13 mM NH_4Cl) conditions (see Materials and Methods for details). mRNA levels of glycosyltransferases compared with those of the housekeeping enzyme, glyceraldehyde-3-phosphate dehydrogenase.

Gawlitzek therefore teaches that while ammonium concentrations may effect terminal galactosylation and sialylation of TNFR-IgG in CHO cells and while ammonium concentrations may be decreased by eliminating glutamine from the culture media of the CHO cells of Gawlitzek (see Figure 2 of Gawlitzek), the reduction of ammonium concentrations provided by elimination of glutamine from the culture media of the CHO cells of Gawlitzek would not effect terminal galactosylation and sialylation of TNFR-IgG in CHO cells.

Gawlitzek therefore teaches away from a method of the claimed invention.

As Gawlitzek is understood to be the only reference cited by the Examiner to allegedly teach or suggest a connection between glutamine concentration and terminal galactosylation and sialylation, the Section 103 rejection should be withdrawn in view of the above demonstration that Gawlitzek teaches that any effect of ammonium on terminal galactosylation and sialylation of TNFR-IgG in CHO cells is related to reduced activity of transferase enzymes at the increased pH caused by the presence of ammonium and that similar transferase enzyme expression and activities are present in CHO cells in the presence and absence of glutamine in the culture media.

None of the remaining cited references (i.e., Wilson (WO 87/04462), Bebbington (U.S. Patent No. 5,891,693), Barsomian (U.S. Patent No. 5,238,821), Brandt (U.S. Patent No. 6,395,484) and Hermitin (U.S. Patent No. 6,096,555)) cure these deficiencies of Gawlitzek. The cited combination of art fails to teach or suggest the claimed invention.

Specifically, Wilson teaches a DNA sequence which encodes the amino acid sequence of CHO glutamine synthetase as well as methods of making and using similar sequences. Wilson fails to teach or suggest any relationship however between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

Bebbington teaches production of a lymphoid cell line which is glutamine independent and the advantage of growing the transformed lymphoid cell line on a

medium containing glutamine followed by growth where glutamine is progressively depleted. See column 1, lines 28-67 of Bebbington, for example. Bebbington does not describe glycosylation patterns of proteins produced from the cell lines or any effect of transformation and growth conditions on same. Bebbington fails to teach or suggest any relationship however between transfection of a glutamine auxotrophic human cell with an enogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek and Wilson or Gawlitzek and Bebbington would not have made the claimed invention obvious.

Barsomian provides enzymes useful to

> "completely deglycosylate an asparagine-glycoprotein or glycopeptides. This is useful for protein sequencing, isoelectric focusing, peptide mapping, and in the two dimensional electrophoresis of glycopeptides and glycoproteins." See column 3, lines 12-17 of Barsomian.

Barsomian does not describe glycosylation patterns of proteins produced from cell lines or any effect of transformation and growth conditions on same. Barsomian fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek, Barsomian and Wilson or Gawlitzek, Barsomian and Bebbington would not have made the claimed invention obvious.

Hermentin provides methods for characterizing the glycolysation of glycoproteins and the in vitro determination of the bio-availability of glycoproteins. Glycoproteins of the examples of Hermentin appear to be produced in CHO and BHK cells. Specific descriptions of the production conditions of the glycoproteins of Hermentin do not appear to be provided.

Hermentin does not any effect of transformation and growth conditions on glycosylation. Hermentin fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek, Barsomian, Hermentin and Wilson or Gawlitzek, Barsomian, Hermentin and Bebbington would not have made the claimed invention obvious.

Brandt provides a method of identification of human cell lines for the production of human proteins by endogenous gene activation. The method of Brandt requires transfecting cells with a DNA construct comprising specific flanking sequences, positive selection marker, optional negative selection marker, optional amplification gene, and a heterologous expression control sequence which is active in human cells. See column 4, lines 18-25 of Brandt. Brandt teaches that producing recombinant target proteins with the "correct glycosylation" is a goal. See column 3, line 24 of Brandt. The examples of Brandt all appear to teach growth of transfected cells (Namalwa cells,

HT1080 cells and HeLa S3 cells) in the presence of glutamine and fetal calf serum. See Example 5 of Brandt.

Brandt does not describe effects of transformation and growth conditions on glycosylation of proteins. Brandt fails to teach or suggest any relationship between transfection of a glutamine auxotrophic human cell with an endogenous DNA sequence encoding a glutamine synthetase and production of glycosylated proteins with an increased sialylation and/or N-glycan charge, as claimed.

The combined teachings of Gawlitzek, Barsomian, Hermentin, Brandt and Wilson or Gawlitzek, Barsomian, Hermentin, Brandt and Bebbington would not have made the claimed invention obvious.

The claims are submitted to be patentable over the combinations of Wilson (WO 87/04462) or Bebbington (U.S. Patent No. 5,891,693) "as evidenced by" Barsomian (U.S. Patent No. 5,238,821) in view of Brandt (U.S. Patent No. 6,395,484) and Gawlitzek (Biotechnology and Bioengineering, Vol. 68, No. 6, June 2000, pp 637-646) and Hermitin (U.S. Patent No. 6,096,555). Entry of the present Amendment is requested. The Section 103 rejection of claims 1, 3-7, 10, 14, 15, 17-24 and 27-29 will be moot upon entry of the present Amendment. Claims 32-35 are submitted to be patentable over the cited combinations of art and withdrawal of the Section 103 rejection of same is requested along with a Notice of Allowance. The Examiner is requested to contact the undersigned, preferably by telephone, in the event anything further is required in this regard.

Respectfully submitted,

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